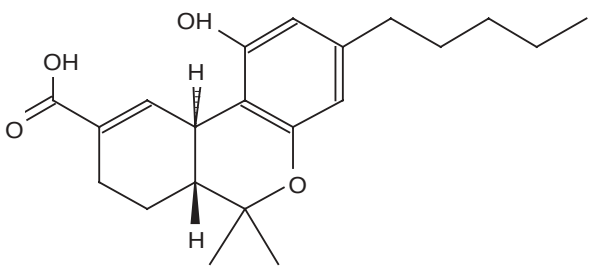
Extraction of THC-COOH from Urine using Strata™ Screen-A

This method is optimized for the extraction and clean-up of 11-Nor-delta-9-Tetrahydrocannabinol-9-Carboxylic Acid (THC-COOH), the primary metabolite of cannabis/marijuana, from urine. Strata Screen-A, a mixed-mode sorbent (C8 + SAX), can retain THC-COOH using a combination of strong anion exchange and secondary non-polar interactions. Contaminants such as urinary salts and non-polar and cationic/amino compounds are effectively removed from the sorbent by water and methanol washes while the anionic THC-COOH is firmly retained by anion exchange. THC-COOH is subsequently eluted using a mixture of water miscible organic solvent and acid modifier in order to neutralize the charged carboxylic acid group. The result is a very clean, concentrated THC-COOH extract.

THC-COOH



Specimen preparation:
Base Hydrolysis: To 2-4mL of urine add 300µL 10M potassium hydroxide. Mix/Vortex. Heat to 60°C for 20 min. Allow solution to cool. Add glacial acetic acid to adjust pH to 4.5-6.5. Add internal standards + 2mL 100mM sodium acetate/methanol solution (95:5, v:v, adjusted to pH 7).

Suggested internal standards for GC/MS: d_3-Carboxy-Δ^9-THC; d_3-THC.

SPE method:

Condition
1) 1mL methanol 2) 1mL 100mM sodium acetate/methanol solution (95:5, v:v adjusted to pH 7)
Load
1) Apply the sample at a rate ≤2mL/min.
Wash/Dry
1) 2mL DI water 2) 2mL methanol/DI water (60:40) 3) Dry column 30-60 sec at full vacuum (>10" Hg) to remove all traces of water.
Elute THC-COOH
1) With the vacuum turned off, apply 2mL hexane/ethyl acetate solution (50:50) + 1% glacial acetic acid. Allow solvent to slowly soak into sorbent for 15-30 sec before applying vacuum. Optimal flow rate of elution solvent is ≤2mL/min.

(Important: The volumes shown are for 100mg sorbent mass. The method can be optimized for smaller or larger bed masses, by adjusting the solvent volumes.)

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Derivatize:
Evaporate to dryness at ≤40°C. Add 50µL ethyl acetate + 50mL BSTFA (with 1% TMCS). Cap, mix/vortex and heat for 20 min at 70°C. Allow the solution to cool. Important: Do not evaporate the BSTFA solution.

BSTFA = N,O-bis(trimethylsilyl)trifluoroacetamide
TMCS = trimethylchlorosilane

Analysis:
Inject 1-2µL derivatized sample onto GC column. (recommended: Zebron ZB-5, 15m x 0.25mm x 0.25µm).
Monitor the following ions (MSD):

Carboxy-Δ^9-THC	d_3-Carboxy-Δ^9-THC	THC	d_3-THC
371	374	303	306 ◀ quantification ions
473	476	315	318
488	491	386	389

Ordering Information:

Order No.	Description	Unit
8B-S019-EAK	Screen-A Tubes (100mg/1mL)	100/Box
8B-S019-FBJ	Screen-A Tubes (200mg/3mL)	50/Box
8E-S019-CGB	Screen-A 96-Well Plate (25mg/well)	2/Box
8E-S019-DGB	Screen-A 96-Well Plate (50mg/well)	2/Box
7EG-G002-11	Zebron ZB-5 (15m x 0.25m x 0.25µm)	1/Box

Extraction Tips!

1. Preparing solutions

100mM sodium acetate
Add 13.6g of sodium acetate to an empty 100mL volumetric flask. Add 90mL DI water and mix. Bring volume up to the mark with DI water. This makes a 1M sodium acetate solution. Dilute 50mL of 1M sodium acetate to 500mL with DI water. Mix.

10M potassium hydroxide
Add 140g of potassium hydroxide to an empty 250mL volumetric flask. Add 150mL DI water to dissolve the solid. Bring the volume up to the mark with DI water.

2. Do not allow the sorbent to dry between the conditioning steps or prior to loading the sample. Excessive drying of the sorbent causes "deconditioning" which may lead to significantly lower and erratic recoveries. To ensure a properly solvated sorbent, apply each solvent immediately after the previous solvent.

3. Always condition the sorbent with the strongest solvent used in the method to ensure the cleanest extraction of target analyte(s). In this method, 100mM sodium acetate/methanol solution is used after methanol.

4. During the wash step, drying the sorbent removes any residual water and will ensure optimal analyte recovery.

5. Any parent THC compound is retained only via hydrophobic interactions with the C8 portion of the sorbent. This interaction can be disrupted with a strong organic solvent. It can be eluted with 2mL hexane/ethyl acetate solution (50:50) prior to the elution solvent recommended for THC-COOH.

Questions? Please contact your Phenomenex Technical Representative.

This method is designed as a convenient starting point for further investigation. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.